Requirements for Submission of gDNA for Exome or Custom Capture Sequencing

Sample Preparation:

1. DNA may be extracted by your method of choice, but the procedure should include or be followed by phenol:chloroform extraction and alcohol precipitation. A protocol is included below.
2. DNA quantitation: we strongly recommend Qubit since it is specific for dsDNA while Nanodrop measurements include contaminating ssDNA, RNA, oligonucleotides and nucleotides.
3. DNA amount: 5 µg
4. Minimum DNA concentration: 20 ng/µl
5. Minimum DNA volume: 10 µl
6. Recommended buffer: 10 mM Tris, 0.1 mM EDTA, pH7.8

Sample Submission:

1. Tube specifications: 1.5 – 2 ml snap cap or screw cap tubes.
2. Sample name must be clearly written on the tube.
3. Submit both by e-mail and with the shipment a completed NISC Sample Submission Form.
4. Submit an agarose gel photo or Bioanalyzer trace for each DNA sample.
5. NISC will confirm concentration using Qubit.

Phenol:Choroform Extraction Protocol for gDNA Clean Up

1. Sample should be in a volume of at least 100 µl for extraction. If necessary dilute with 10 mM Tris, 1 mM EDTA, pH 7.8.
2. Add an equal volume of fresh phenol:choroform:isoamyl alcohol (25:24:1) (Invitrogen cat. no. 15593-031).
3. Vortex vigorously for 30 sec. Centrifuge at top speed in a microcentrifuge* for 5 min.
4. Carefully transfer the upper layer to a fresh microfuge tube. Add an equal volume of chloroform.
5. Vortex vigorously for 30 sec. Centrifuge at top speed in a microcentrifuge for 5 min.
6. Carefully transfer the upper layer to a fresh microfuge tube. Add $1/10^{th}$ volume of 3 M NaOAc and 2.5 volumes of ethanol (95-100%). Mix well and let set in ice for 15 min.
7. Centrifuge at top speed in a microcentrifuge for 30 min.
8. Carefully remove supernatant. Recentrifuge for 5 sec and carefully pipet off remaining liquid not disturbing the pellet. Hint: orienting the hinge of the

tube upwards before centrifugation puts an invisible DNA pellet on the upper wall of the tube bottom so one can avoid it when pipetting.

9. Add 0.5 ml of 70 % ethanol. Centrifuge at top speed in a microcentrifuge for 5 min.
10. Carefully remove supernatant. Recentrifuge for 5 sec and pipet off remaining liquid.
11. Allow to air dry 5 min. DO NOT OVER DRY GENOMIC DNA – it can be very difficult to redissolve.
12. Dissolve DNA pellet in volume of sterile 10 mM Tris, 0.1 mM EDTA, pH7.8 to bring concentration to ~100 μg/ml.

* Phase Lock Gel Tubes (5 Prime cat. no. 2302800) can be helpful in extraction. See https://www.5prime.com/products/nucleic-acid-purification/organic-nucleic-acid-extraction/phase-lock-gel-.aspx for details.